Title:

Newly Identified Nematodes from Mono Lake Exhibit Extreme Arsenic Resistance

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Summary

Extremophiles have much to reveal about the biology of resilience, yet their study is limited by sampling and culturing difficulties [1-3]. The broad success and small size of nematodes make them advantageous for tackling these problems [4-6]. We investigated the arsenic-rich, alkaline, and hypersaline Mono Lake (CA, USA) [7-9] for extremophile nematodes. Though previously described to contain only two animal species (brine shrimp and alkali flies) in its water and sediments [10], we report the discovery of eight nematode species from the lake, including microbe-grazers, parasites, and predators. Thus, nematodes are the dominant animals of Mono Lake in species richness. Phylogenetic analysis suggests the nematodes originated from multiple colonization events, which is striking given the young history of extreme conditions at Mono Lake [7, 11]. One species, *Auanema* sp., is new, culturable, and survives 500-times the human lethal dose of arsenic. Comparisons to two non-extremophile sister species [12] reveals that arsenic resistance is a common feature of the genus and a preadaptive trait that likely allowed *Auanema* to inhabit Mono Lake. This preadaptation may be partly explained by a variant in the gene *dbt-1* shared with some *C. elegans* natural populations and known to confer arsenic resistance [13]. Our findings expand Mono Lake's ecosystem from two known animal species to ten, and provide a new system for studying arsenic resistance. The dominance of nematodes in Mono Lake and other extreme environments, and our findings of preadaptation to arsenic raise the intriguing possibility that nematodes are widely pre-adapted to be extremophiles.

Keywords: Mono Lake, Arsenic, Extremophiles, Nematodes, Preadaptation, Resilience

Results

Nematodes were isolated from Mono Lake

Mono Lake covers 13 miles east to west, and 8 miles north to south, with variable levels of human activity and environmental conditions characterizing its lakeshores. To survey for animal life in the sediments of Mono Lake, we collected soil from three different sites to sample across these conditions: Site A (Pristine Beach) in the northeast, Site B (Navy Beach) in the south, and Site C (Old Marina) in the west (**Figure 1A, S1**). Site A is inaccessible by vehicles and possesses the least observable human activity of the three sites. Site B attracts the most tourists and contains tufa structures. Site C has a rocky shore with small tufa structures. We found *Artemia monica* brine shrimp in the lake water and upper lake sediment, larvae of *Ephydra hians* alkali flies in the lake sediment, and adult flies on the lakeshores.

At each site, we collected soil from the dry zone, tide zone, and in-lake—defined by their distances to the shore (**Table S1, Figure S1B**). By extracting samples on-site and in the laboratory using Baermann funnels, we isolated live nematodes from all three sites (**Figure S1D**). In contrast to the sediments, we did not find nematodes in the water columns. Nematodes, brine shrimp, and alkali flies were the only animals isolated from the samples, highlighting the harshness of the environment. To rule out the possibility that nematodes were only present at the lake as a result of environmental contamination, we sampled the secluded Pristine Beach (Site A, **Figure S1A**). We isolated nematodes from this site in the summers of 2016 and 2017, suggesting the lake hosts a regular population of nematodes (**Table S1**).

To understand the environmental conditions that support nematode growth, we measured the pH and soil salinity of our samples (**Table S1**). The average pH of the samples ranged between 9-10 across the three sites. In contrast, the salinity of the samples varied highly (**Figure S1C**). This chemical analysis aligns with the geography of Mono Lake: Site A lacks any entry point of freshwater streams and is the most chemically extreme of the three sites we sampled (**Figure S1C**). Nevertheless, Site A hosts nematodes in the tide zone and in-lake (**Table S1**), suggesting nematodes have adapted to thrive at even these extreme niches of the lake.

Mono Lake nematodes represent diverse clades and lifestyles

From the several distinct nematodes we found, we characterized eight species by DNA analysis (species a-h, **Figure 1B**). One species was isolated in-lake in Site A, seven from Site B, and three from Site C. In 2017, we found two of the species (e and f) again at different locations (Site B tide zone in 2016 and Site C dry zone in 2017) (**Figure 2A**), further suggesting nematodes form regular and widespread populations in the lake.

We identified the nematode species using 28rDNA large subunit (LSU) and 18rDNA small subunit (SSU) signatures. This analysis indicated that three of the isolated nematodes are known species, while five are previously un-sequenced species (**Figure 2A, S2C**). The isolates are distributed across the phylogeny of Nematoda (**Figure 2B**), with known species *Mononchoides americanus* (species c) and *Diplogaster rivalis* (d) in Clade V, and *Prismatolaimus dolichurus* (f) in Clade II. Two of the un-sequenced species, *Auanema* sp. (a) and *Pellioditis* sp. (b), belong to Clade V. We assigned the other three un-sequenced species to families instead of genera because they lack phylogenetically close species: species in Mermithidae (e, Clade I) and species in Monhysteridae (g and h, between Clade II and III) (**Figure S2C**). Taken together, the diverse distribution across the phylum Nematoda suggests Mono Lake was colonized by nematodes multiple times and in independent events.

We identified several mouth structures within the eight species (**Figure S2**), including grinders (*Auanema* sp., *Pellioditis* sp., species in Monhysteridae), teeth (*M. americanus* and *D. rivalis*), and a long esophagus with small teeth-like structures (*P. dolichurus*). The mouth structure of a nematode is an indicator of its feeding style [14], and from this analysis we predict that *Auanema* sp., *Pellioditis* sp., and the species in Monhysteridae are microbe-feeders, and that *M. americanus*, *D. rivalis*, and *P. dolichurus* are predators (**Table S2**). In addition, species in Mermithidae (e) belongs to a family whose members are parasites of arthropods [15, 16], thus species e is likely a parasite of the brine shrimp or alkali flies living in the lake. Taken together, our data show that the ecosystem of Mono Lake is more complex than previously thought, and establish nematodes as the dominant animals within Mono Lake in species richness and diversity (**Figure 2C**).

Auanema sp. is a new and culturable species

Few animal extremophiles have been cultured in the laboratory, such as tardigrades and killifish [17, 18]. Thus, we sought to culture Mono Lake nematodes to better understand animal resilience. Strikingly, one of the species we isolated, *Auanema* sp., was readily culturable using established *C. elegans* methods [19] (**Figure 3A**). We generated two strains of *Auanema* sp. from different sites in Navy Beach, PS8402 and PS8403 (**Table S2**), which could be frozen into stocks and viably thawed. Under these conditions, *Auanema* sp. has a reproductive lifespan of 2.5-3 days at 22.5°C, comparable to *C. elegans*.

The *Auanema* genus contains five previously known species that were found in diverse habitats, but none from an extreme environment [12] (**Figure S2B**). *Auanema* sp. shares features with its closest relatives, *A. rhodensis* and *A. freiburgensis* [12], such as possessing three sexes: hermaphrodites, males, and females (**Figure S3H-Q**). However, *Auanema* sp. demonstrates unique typological, biological, and phylogenetic traits. For instance, the arrangement of genital papillae in *Auanema* sp. males is unique in the genus (**Figure S3L-N**). *Auanema* sp. also uniquely live-birth their larvae after hatching the embryos in their uterus (viviparity) (**Figure 3B, S3I-J**). Live-birthing is a common feature of extremophile nematodes suggested to serve an adaptive role in extreme environments [20, 21], and may therefore be a strategy *Auanema* sp. uses to protect its progeny from the harsh conditions of Mono Lake.

Furthermore, *Auanema* sp. is distinguished from its genus as it does not demonstrate the tube nictation behavior typical of *Auanema* [12]. Finally, *Auanema* sp. shares less than 98% sequence identity with its closest relative, *A. rhodensis* (89% LSU, 96% SSU) (**Figure S2C**). On the basis of these unique traits, we conclude that *Auanema* sp. is a new species (**Data S1**).

Auanema sp. is arsenic-resistant

Mono Lake possesses high concentrations of arsenic, in primarily As(III) arsenite and As(V) arsenate forms [8]. We exposed *Auanema* sp. and the laboratory nematode *C. elegans* to increasing concentrations of As(III) and As(V) liquid solutions to

characterize their resistance to arsenic. *C. elegans* is an informative reference as it has been reported to survive 60-times the human lethal dose of arsenic ([13] and STAR Methods).

We confirmed that exposure to water-only led to near 100% survival of the two species within the time window of the assay (*Auanema* sp. range = 90 to 100%, *C. elegans* range = 93 to 100%) (**Figure S4C**). In 1.5 mM As(III), we observed significantly higher survival in *Auanema* sp. versus *C. elegans* at 2.5 hours (*Auanema* sp. mean survival = 82%, *C. elegans* mean = 25%, $q < 0.01$ by permutation test) (**Figure 4A**). We observed a similar difference at 5 hours of exposure (*Auanema* sp. mean = 35%, *C. elegans* mean = 7%, $q < 0.05$), but at 7 hours both nematodes exhibited indistinguishably low survival (*Auanema* sp. mean = 24%, *C. elegans* mean = 7%, not significant). Similar results were obtained at an increased concentration of 4.5 mM As(III) (**Figure S4A**).

Strikingly, in 10 mM and 30 mM As(V), *Auanema* sp. exhibited high survival over the 7 hours of the assay (**Figure 4A, S4B**). For instance, at 7 hours in 30 mM As(V), *Auanema* sp. demonstrated a mean survival rate of 79% compared to 16% in *C. elegans* ($q < 0.005$). Therefore, *Auanema* sp. can withstand high concentrations of As(V)—approximately 500-times the human lethal limit—for a prolonged period of time. This strong tolerance of As(V) is consistent with the fact that *Auanema* sp. was isolated from near the surface of the tide zone, where As(V) is reported to be the dominant arsenic compound [22]. Altogether, these results suggest extreme arsenic resistance allows *Auanema* sp. to survive in Mono Lake.

Arsenic resistance is a preadaptive trait in *Auanema*

We further compared the arsenic resistance of *Auanema* sp. to its sister species *A. freiburgensis* and *A. rhodensis*, under quick-killing conditions of 2.5 hour exposure to 1.5 mM As(III). Strikingly, *A. freiburgensis* and *A. rhodensis* demonstrated slightly higher survival than *Auanema* sp. (*Auanema* sp. mean = 86%, *A. freiburgensis* mean = 99%, $q < 0.05$; *A. rhodensis* mean = 97%, $q < 0.05$) (**Figure 4B**). Therefore, despite being isolated from non-extreme environments, *A. freiburgensis* and *A. rhodensis* demonstrate strong resilience to arsenic. This data indicates arsenic resistance is a

common feature of the *Auanema* genus and a preadaptive trait that likely allowed *Auanema* to inhabit Mono Lake.

Conceivably, the strong resistance of the three *Auanema* species could be due to novel genes or natural variants in existing genes. One natural variant that affects arsenic sensitivity in *C. elegans* is the missense variant DBT-1(C78S). This variant in the branched-chain amino acid metabolism protein occurs naturally in the Hawaiian strain of *C. elegans* and improves the strain's brood size in arsenic [13]. We sequenced *dbt-1* from the three *Auanema* species and observed that they all share the serine variant with *C. elegans* Hawaii (**Figure 4C, S4D**).

We tested arsenic resistance in *C. elegans* Hawaii, and indeed Hawaii demonstrated higher survival than laboratory wildtype, *C. elegans* Bristol, when exposed to 1.5 mM As(III) for 2.5 hours (Hawaii mean = 65%, Bristol mean = 35%, $q < 0.005$) (**Figure 4B**). However, survival in *C. elegans* Hawaii was significantly lower than in *A. freiburgensis* (mean = 99%, $q < 0.005$), *A. rhodensis* (mean = 97%, $q < 0.001$), and *Auanema* sp. (mean = 86%, $q < 0.01$). Therefore, preadaptation to arsenic in *Auanema* might be partly explained by the serine variant in DBT-1, but other factors likely contribute to the higher resistances of the *Auanema* species compared to *C. elegans* Hawaii.

Discussion

While Mono Lake was previously thought to only contain two animal species (brine shrimp and alkali flies) in its waters and sediments, we report that nematodes are widespread throughout the lake and are the dominant animals in species richness and diversity. We isolated nematodes from across the lake in two consecutive years and uncovered multiple niches where they can thrive. We identified eight nematode species representing diverse clades and lifestyles (such as microbe-grazing, parasitism, and predation), indicating multiple colonization events took place in Mono Lake. The young history of extreme conditions at Mono Lake [7, 11] makes this particularly surprising and suggests nematodes in the naïve lake may have been pre-adapted for subsequent changes.

Due to the high level of protection of Mono Lake, our sampling was likely far from saturated. Indeed, when we isolated the same species in subsequent years, we did not find them in the same site. Our unsaturated sampling may also explain why the nematodes we observed in low abundance the first year (*e.g. D. rivalis*) were not observed in the second year. It is therefore likely that the diversity of nematodes in Mono Lake is richer than what we have observed.

One new species, *Auanema* sp., is culturable and demonstrates viviparity and strong arsenic resistance—two traits that likely increase its fitness in Mono Lake. In addition, its non-extremophile sister species *A. freiburgensis* and *A. rhodensis* are arsenic resistant as well, indicating preadaptation to arsenic across the vast geographic distances between the species (Germany, France, USA [12]). We speculate that this preadaptation is related to their ecology. Previous *Auanema* species were isolated from rich soils and dung, which can contain high concentrations of phosphate [23]. Since arsenic uptake occurs adventitiously via phosphate transporters [24], it is conceivable that adaptation to high levels of phosphate in the environment could lead to increased arsenic resistance as well.

We have identified Mono Lake as another harsh environment where nematodes survive, similar to the Antarctic desert, deep sea, and subterranea [4, 5, 25]. The broad success of nematodes in inhabiting extreme environments suggests strong preadaptation throughout the phylum. This is consistent with the dominance of

nematodes in Mono Lake, despite the lake's young history of extreme conditions; with preadaptation to arsenic in *Auanema* across vast distances; and with the lesser but significant arsenic resistance in *C. elegans* [13]. Our findings therefore highlight the intriguing possibility that nematodes may be widely pre-adapted to be extremophiles.

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Author Contributions

Conceptualization, P.-Y.S., J.S.L., R.S., A.S., P.W.S.; Formal Analysis, P.-Y.S., J.S.L., R.S., N.K., A.P.-d.S., A.S; Investigation, P.-Y.S., J.S.L., R.S., N.K., A.P.-d.S., J.M.B., A.S.; Resources, A.P.-d.S., E.G., A.S.; Writing – Original Draft, P.-Y.S., J.S.L.; Writing – Review & Editing, P.-Y.S., J.S.L., R.S., N.K., E.G., A.S., P.W.S.; Visualization, P.-Y.S., J.S.L., R.S., N.K.; Supervision, A.S., P.W.S.; Funding Acquisition, P.W.S.

Declaration of Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figure Legends

Figure 1. Eight nematode species were isolated from Mono Lake. (A) Locations of the three sampling Sites A, B and C around Mono Lake. Samples were collected in August 2016 and June-July 2017. **(B)** Morphology of nematodes isolated from the three sites, under low magnification. See also **Figure S1**, **Table S1** and **Table S2**.

Figure 2. Classification of nematode phylogeny and lifestyle. (A) Species were identified by 28S and/or 18S rRNA. Year of collection and (sample site) are indicated. Colors denote the years. Footnotes: 1, identified by LSU; 2, identified by SSU; 3, Clade I-V system [26]; 4, Clade 1-13 system [27]. Positions between Clade II and III are indicated with parentheses. **(B)** Bayesian phylogenetic tree of the isolated nematodes, based on SSU sequences. Red text: Mono Lake nematodes. Numbers: posterior probabilities. **(C)** Model of ecological relationships in Mono Lake. Arrows point to consumed organisms. Dashed arrows: predicted by nematode morphology, phylogeny, and sampling. Red: *Auanema* sp. Fill colors: different phyla. Not drawn to scale. See also **Figure S2** and **Table S2**.

Figure 3. *Auanema* sp. is culturable and demonstrates viviparity. (A) Adult *Auanema* sp. hermaphrodite grown in the laboratory. Individual is from a line (PS8402) that was passaged singly for more than nine generations. Hatched larva indicated by yellow outline. **(B)** 100x image of an adult hermaphrodite with one hatched larvae in its uterus. Ph: pharynx of the larva, Oc: oocyte, D: dorsal, P: posterior. See also **Figure S3** and **Data S1**.

Figure 4. Arsenic resistance is a common feature of the *Auanema* genus and a preadaptive trait. (A) Survival curves of *Auanema* sp. (blue) and *C. elegans* (orange) adults in arsenic solutions. Points: individual populations (average 31 animals). Bars: standard error of the mean. Statistics: non-parametric permutation test at each time point. "*" $q < 0.05$, "**" $q < 0.01$, "***" $q < 0.005$. **(B)** Survival of *C. elegans* Bristol, *C. elegans* Hawaii, *Auanema* sp., *A. freiburgensis*, and *A. rhodensis* adults after 2.5 hour

exposure to 1.5 mM As(III). Points: individual populations (average 35 animals). Bars: bootstrapped mean and 95% CI. Letters: groups statistically different from each other by pairwise permutation tests and *q*-value cutoff of 0.05. **(C)** Alignment of DBT-1 protein sequence surrounding the Cys/Ser variant position (yellow). See also **Figure S4**.

STAR Methods

Lead contact and materials availability

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Paul W. Sternberg (pws@caltech.edu).

Experimental model and subject details

Maintenance

All animals were grown using standard *C. elegans* culturing protocol with *Escherichia coli* strain OP50 as a food source [19]. That is, animals were cultured on 6 cm plates containing nematode growth media (NGM: 3 g NaCl, 2.5 g bacto-peptone, 17 g bacto-agar, 975 mL distilled H_2O, autoclaved followed by adding 1 mL cholesterol (5 mg/mL in ethanol), 1 mL of 1 M $CaCl_2$, 1 mL of 1 M $MgSO_4$, and 25 mL of 1 M KPO_4 buffer (pH 6.0) sequentially) seeded with approximately 50 µL OP50 liquid culture (single colony OP50 grown overnight in LB broth at 37°C and stored at 4°C).

Caenorhabditis elegans wild-type strain N2 (Bristol), *C. elegans* natural isolate CB4856 (Hawaii), *Auanema freiburgensis* (APS7), *A. rhodensis* (APS4), and *Auanema* n. sp. (PS8402 and PS8403) were used as experimental models for this study. *Auanema* sp. PS8402 was obtained from a single adult on August 16, 2016 from sample B14, and was passaged singly for more than nine generations. *Auanema* sp. PS8403 was obtained from a single adult on August 18, 2016 from sample B8. All animals were maintained at 20°C (*A. rhodensis*) or 22.5°C. Animals were transferred to new plates approximately every three days.

Freezing

Auanema sp. was frozen using Trehalose-DMSO freezing solution (personal communication with Dr. Kevin F. O'Connell). Briefly, *Auanema* sp. from freshly starved plates were washed off with M9 buffer (3 g KH_2PO_4, 6 g Na_2HPO_4, 5 g NaCl and 1 mL 1 M $MgSO_4$ in 1L ddH_2O), washed once, re-suspended with Trehalose-DMSO freezing buffer (15.1 g Trehalose (Fisher BioReagents, PA, Cat# BP2687-25) and 17.7 mL DMSO in 500 mL M9 buffer), and transferred to cryogenic vials. The vials were stored in

an -80°C freezer after 30 minutes incubation at room temperature.

Method details

Sites and sampling

Soil and water samples were collected from three sites around Mono Lake (**Figure 1, S1A**) in August 2016, June 2017, and July 2017. Site A, which we named Pristine Beach, (38° 3′ 27.91″ N, 119° 1′ 50.66″ W), Site B at Navy Beach (37° 56′ 21.90″ N, 119° 1′ 25.93″ W), and Site C at Old Marina (37° 59′ 12.80″ N, 119° 8′ 18.70″ W).

At each site, soil samples were collected from inside the lake, tide zone, and dry zone, with each sample ranging from 15 g to 375 g in weight. The total number of samples collected from each site was: 25 from Site A (9 in 2016 and 16 in 2017), 34 from Site B (19 in 2016 and 15 in 2017), and 22 from Site C (7 in 2016 and 15 in 2017). The sampling permits were issued to Amir Sapir by the California Fish and Wildlife Department (SCP-13436) and from the Californian State Parks Department. All of the sample information including location, pH, salinity, and the presence of nematodes is listed in **Tables S1 and S2**.

Soil salinity and pH measurement

Each soil sample was mixed with Milli-Q water in a 1:2 ratio (weight:volume) for salinity and pH measurements [28]. Soil salinity was estimated by measuring the conductivity with two meters: Orion conductivity meter model 126 (for 2016 samples) and TPS WP-81 conductivity meter (for 2017 samples). Soil pH was measured using VWR pH meter model 8015.

Nematode isolation and species identification

Nematodes were isolated directly from the soil samples either on-site or in the laboratory as follows. On-site: isolations were performed by mixing soil samples with Mono Lake water in 10 cm plates, and the materials were manipulated using Irwin loops under a dissecting microscope. In lab: soil samples were extracted overnight by the Baermann funnel method [29]. Specifically, Baermann funnel set-ups with a drain strainer at the mouth of the funnel and a clamped rubber tube attached to the bottom of

the funnel were held onto a retort stand by a ring clamp. Extractions were performed by placing approximately 20 g of soil sample on caligraphy paper set on top of the funnel, adding Milli-Q water until the sample was submerged, soaking overnight, and collecting the first drops of water from the bottom of the funnel by releasing the tube clamp.

The isolated nematodes were further identified by morphology and molecular signatures. Morphological observations were made on live specimens, anesthetized using 20 mM sodium azide, on 2% agarose slides under a Nomarski microscope. For molecular analysis, individual worm lysate was prepared in worm lysis solution (100 μL DirectPCR lysis reagent (Viagen Biotech), 10.5 μL proteinase K (10 mg/mL) and 5 μL 1M DTT). The gene fragments of ribosome large subunit (LSU) 28rDNA and small subunit (SSU) 18rDNA were amplified [30, 31] and sequenced using the primers D2A, D3B, SSU18A, and SSU26R in the **Key Resources Table**.

MEGA7 was used to build phylogenetic trees from the resulting sequences [32]. The trees were estimated by using Maximum Likelihood analysis and 1,000 bootstrap replicates, and the species identification was done with a General Time Reversible (GTR) + I + G model [33]. Trees with the highest log likelihood were selected. Initial trees for the heuristic search were obtained by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with a superior log likelihood value. Isolated nematodes were considered a potentially new species when they exhibited <98% sequence similarity compared to its nearest neighbor [34, 35]. Subsequent Bayesian phylogenetic analysis was performed using MrBayes 3.2.6 [36], using a GTR + I + G model and MCMC analysis with two runs and eight chains over two million generations.

Survival assay

As(III) and As(V) solutions were prepared by dissolving sodium (meta)arsenite (Sigma-Aldrich, MO, Cat# S7400) and sodium arsenate dibasic heptahydrate (Sigma-Aldrich, MO, Cat# S9663) in Milli-Q water, respectively. Adult animals were washed with Milli-Q

water 4 times and transferred to 12-well tissue culture plates (Corning, NY) containing 0.9 mL As(III) or As(V) solution per well. Wells averaged 32 animals, ranging from 5 to 74. Plates were incubated at 20°C, and surviving animals were counted by their physiology and eyelash touch-provoked movement. Specifically, animals were counted as dead if they met at least two of the following criteria: lack of spontaneous or touch-evoked swimming, grey discoloration, tissue degradation, body wall rupturing, and stiff, especially straight posture.

For survival curve assays, H_2O-only, 1.5 mM As(III), 4.5 mM As(III), 10 mM As(V), and 30 mM As(V) was used to treat the animals, and survival was counted at 1, 2.5, 5, and 7 hours. For comparisons of survival in *C. elegans* Bristol, *C. elegans* Hawaii, *A. freiburgensis*, *A. rhodensis*, and *Auanema* sp., 2.5 hour exposure to 1.5 mM As(III) was used. This condition was chosen based on the survival curves (**Figure 4**, **Figure S4**) to maximize the dynamic range between *C. elegans* Bristol and *Auanema* sp., and to minimize time the species spent in water without food. Statistical analysis (non-parametric, pairwise permutation test) was performed using the rcompanion [37] and coin [38] packages in R. Additional analysis was performed using linear mixed effect models (dependent variable = percent survival, fixed effects = species and time, random intercept term = individual populations) followed by Type II Wald chi-square tests, which produced similar conclusions as the permutation tests.

Relative arsenic resistance

The relative arsenic resistance of *C. elegans* and *Auanema* sp. versus humans was calculated by comparing to the human lethal dose of arsenic trioxide, which is 1-3 mg/kg [39, 40]. Assuming an average human density of 1.051 kg/L [41], the human acute lethal dose is approximately 0.016 mM. The previously reported survival of *C. elegans* in 1 mM arsenic trioxide for several days (at least two generations) [13] corresponds to a relative resistance of 60-times the human lethal dose. Similarly, the survival of *Auanema* sp. in 30 mM As(V) corresponds to 1,800-times the human lethal dose, but is better approximated as 200- to 900-times the lethal dose, or an average of 500-times, since trivalent arsenic is generally 2-10 times more toxic than pentavalent arsenic [42,

43].

DBT-1 alignment

The DBT-1 sequences of *C. elegans* Bristol and Hawaii were obtained from WormBase and CeNDR [44]. DBT-1 homologs in *A. freiburgensis* and *A. rhodensis* were identified by reciprocal BLAST to their predicted coding sequences [45]. One putative homolog was found in both species and confirmed by alignment. The DBT-1 sequence in *Auanema* sp. PS8402 was obtained by RNA extraction, reverse-transcription to cDNA, and PCR amplification followed by sequencing: RNA was purified from mixed stages of PS8402 grown on five standard NGM plates using the Qiagen RNeasy kit (Cat No. 74104). cDNA was made using the Maxima H Minus First Strand cDNA Synthesis Kit (Cat No. K1681). PCR was performed using primers based on sequences conserved between *A. freiburgensis* and *A. rhodensis dbt-1* (see **Key Resources Table**). Sequencing produced 438 nucleotides that were translated to a 146 amino acid fragment of the protein, spanning the Cys/Ser natural variant site. Alignment was performed using Clustal [46].

Quantification and statistical analysis

Maximum Likelihood analysis was performed for species identification (**Figure 2A and S2C**) using aligned SSU and LSU sequences, with 1,000 bootstrap replicates. Analysis was performed in MEGA7 [32], using a General Time Reversible (GTR) + I + G model to model nucleotide substitutions as involving different rates of transitions, transversions, and nucleotide frequencies, with the addition of invariable sites and different rates of variability across sites [33].

Bayesian analysis was performed to model phylogenetic relationships across Nematoda (**Figure 2B**) using aligned SSU sequences. Analysis was performed using MrBayes 3.2.6 [36]. A GTR + I + G model and MCMC analysis (two runs and eight chains over two million generations) was used to model nucleotide substitutions. Posterior probabilities are indicated by numbers in **Figure 2B**.

Pairwise permutation tests, which are non-parametric and do not assume normality of the data, were used to analyze the arsenic survival experiments at each time point. Testing was performed using the rcompanion [37] and coin [38] packages in R, using a *q*-value cutoff of 0.05. Calculated means and *q*-values are presented in the **Results**. The numbers of trials that were assayed are indicated by points in **Figure 4 and S4**, and the average number of animals assayed are stated in the **Figure Legends** and **STAR Methods** *Survival Assay* section.

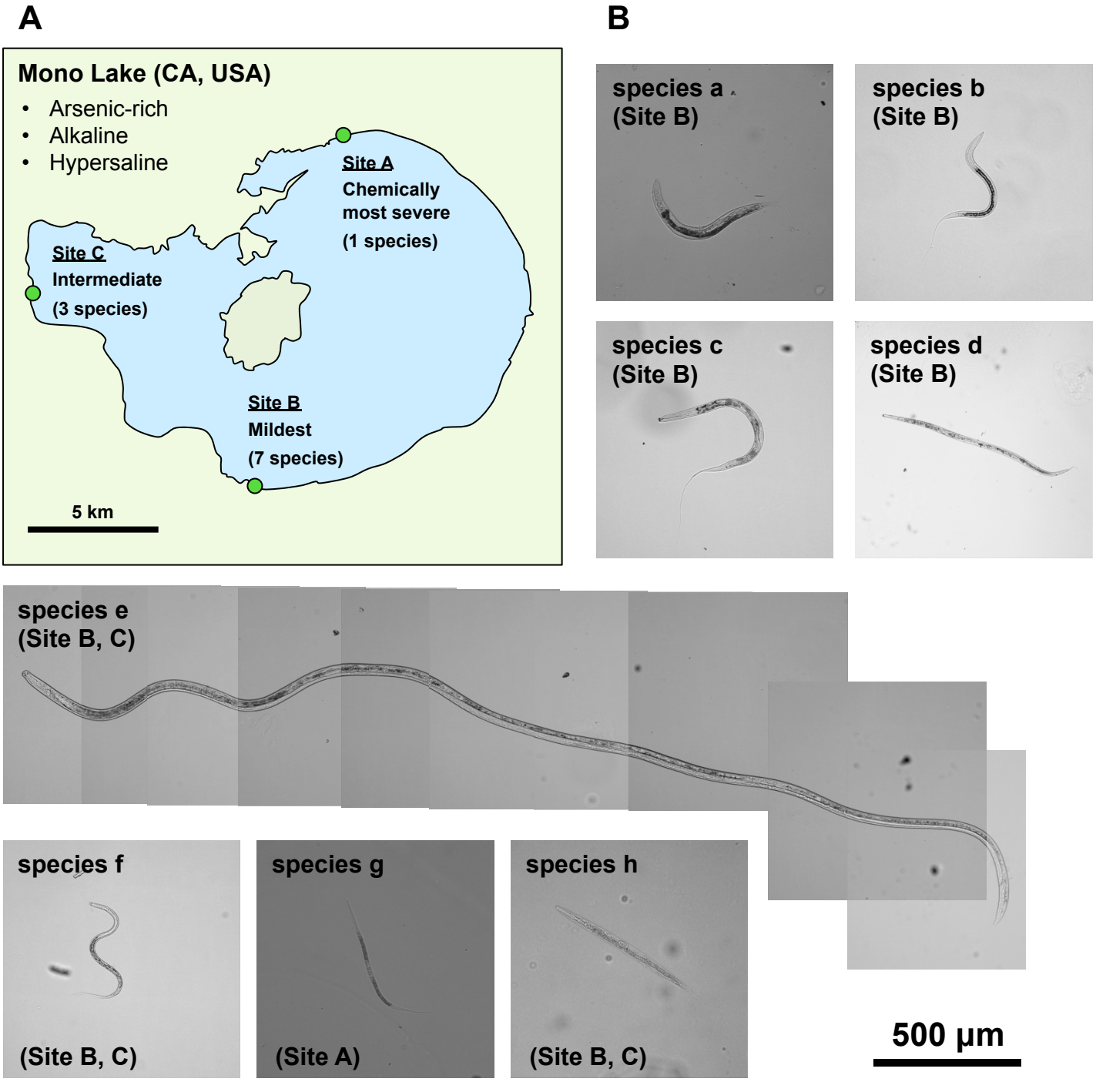
Data and code availability

The LSU and SSU rDNA sequences generated during this study are available at GenBank, under the accession numbers listed in the **Key Resource Table**.

Key Resources Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and Virus Strains		
Escherichia coli: Strain OP50	*Caenorhabditis* Genetics Center	OP50
Chemicals, Peptides, and Recombinant Proteins		
Trehalose	Fisher BioReagents	Cat# BP2687-25
DirectPCR lysis reagent	Viagen Biotech	Cat# 102-T
sodium (meta)arsenite	Sigma-Aldrich	Cat# S7400
sodium arsenate dibasic heptahydrate	Sigma-Aldrich	Cat# S9663
RNeasy Mini Kit	Qiagen	Cat# 74104
Maxima H Minus First Strand cDNA Synthesis Kit with dsDNase	Thermo Scientific	Cat# K1681
Deposited Data		
Auanema sp. LSU rDNA	This paper	GenBank: MK932839
Auanema sp. SSU rDNA	This paper	GenBank: MK932834
Pellioditis sp. LSU rDNA	This paper	GenBank: MK932840
Pellioditis sp. SSU rDNA	This paper	GenBank: MK932835
Mononchoides americanus LSU rDNA	This paper	GenBank: MK940279
Mononchoides americanus SSU rDNA	This paper	GenBank: MK937614
Diplogaster rivalis LSU rDNA	This paper	GenBank: MK940280
Diplogaster rivalis SSU rDNA	This paper	GenBank: MK937615
species in Mermithidae LSU rDNA	This paper	GenBank: MK932841
species in Mermithidae SSU rDNA	This paper	GenBank: MK932836
Prismatolaimus dolichurus LSU rDNA	This paper	GenBank: MK940281
species g in Monhysteridae SSU rDNA	This paper	GenBank: MK932837
species h in Monhysteridae SSU rDNA	This paper	GenBank: MK932838
Experimental Models: Organisms/Strains		
Caenorhabditis elegans: Strain N2: Bristol	Caenorhabditis Genetics Center	N2
C. elegans: Strain CB4856: Hawaii	Caenorhabditis Genetics Center	CB4856
Auanema sp.: Strain PS8402	This paper	PS8402
Auanema sp.: Strain PS8403	This paper	PS8403
A. rhodensis: Strain APS4	[12]	APS4
A. freiburgensis: Strain APS7	[12]	APS7
Oligonucleotides		
LSU-F: 5'–ACAAGTACCGTGAGGGAAAGTTG–3'	[31]	D2A
LSU-R: 5'–TCGGAAGGAACCAGCTACTA–3'	[31]	D3B
SSU-F: 5'–AAAGATTAAGCCATGCATG–3'	[30]	SSU18A
SSU-R: 5'–CATTCTTGGCAAATGCTTTCG–3'	[30]	SSU26R
Auanema dbt-1-F: 5'–GAAGGAATTGCTGAAGTGCAAG–3'	This paper	N/A
Auanema dbt-1-R: 5'– GCNGGAGANGCTCTGATATTC–3'	This paper	N/A

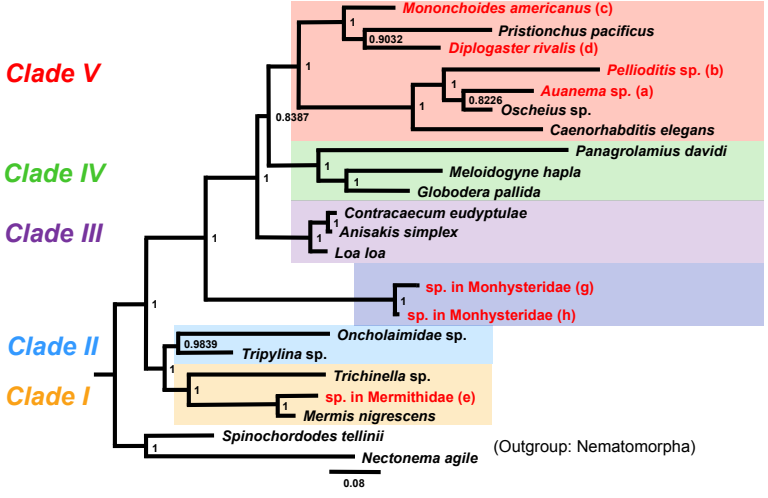
Software and Algorithms		
MEGA7	[32]	https://www.megasoftware.net/
Clustal Omega	EMBL-EBI	https://www.ebi.ac.uk/Tools/msa/clustalo/
R	The R Foundation	https://www.r-project.org
rcompanion (R package)	CRAN	http://rcompanion.org/
coin (R package)	CRAN	http://coin.r-forge.r-project.org/
Other		
Conductivity meter	Orion	Model 126
Conductivity meter	TPS	Model WP-81
pH meter	VWR	Model 8015


A
Mono Lake (CA, USA)
• Arsenic-rich
• Alkaline
• Hypersaline
Site A
Chemically most severe
(1 species)
Site C
Intermediate
(3 species)
Site B
Mildest
(7 species)
5 km
B
species a (Site B)
species b (Site B)
species c (Site B)
species d (Site B)
species e (Site B, C)
species f
(Site B, C)
species g
(Site A)
species h
(Site B, C)
500 µm

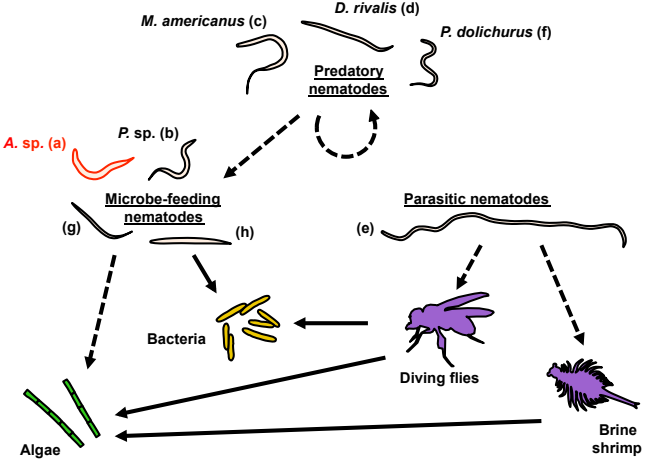
A

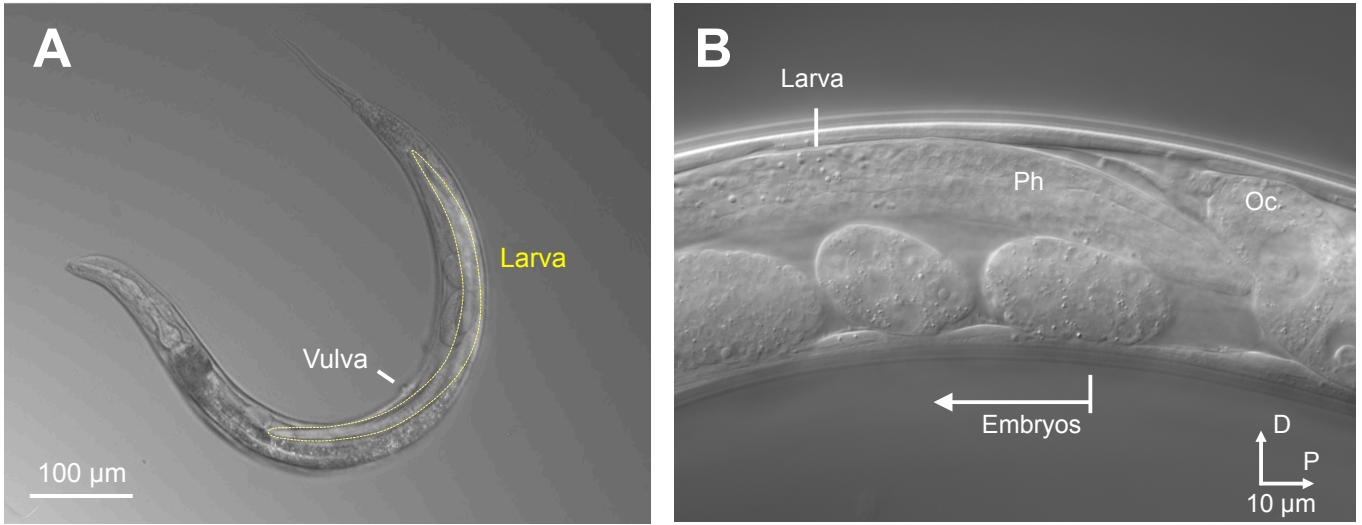
Species	Clade		Lifestyle	Site A		Site B		Site C		
	Blaxter[3]	Helder[4]		tide zone	lake	dry zone	tide zone	dry zone	tide zone	lake
a. *Auanema* sp.[1,2]	V	9	Microbe-feeding				2016 (B8) 2016 (B14)			
b. *Pellioditis* sp.[1,2]	V	9	Microbe-feeding			2016 (B20)	2016 (B9)			
c. *Mononchoides americanus*[1,2]	V	9	Predatory			2016 (B20)	2016 (B7) 2016 (B9)			
d. *Diplogaster rivalis*[1,2]	V	9	Predatory				2016 (B8)			
e. species in Mermithidae[1,2]	I	2	Parasitic				2016 (B9)	2017 (C131) 2017 (C133)		
f. *Prismatolaimus dolichurus*[1]	II	1	Predatory				2016 (B7)	2017 (C130)		
g. species in Monhysteridae[2]	(II, III)	5	Microbe-feeding		2016 (A9)					
h. species in Monhystreidae[2]	(II, III)	5	Microbe-feeding				2016 (B8)	2016 (C7)		

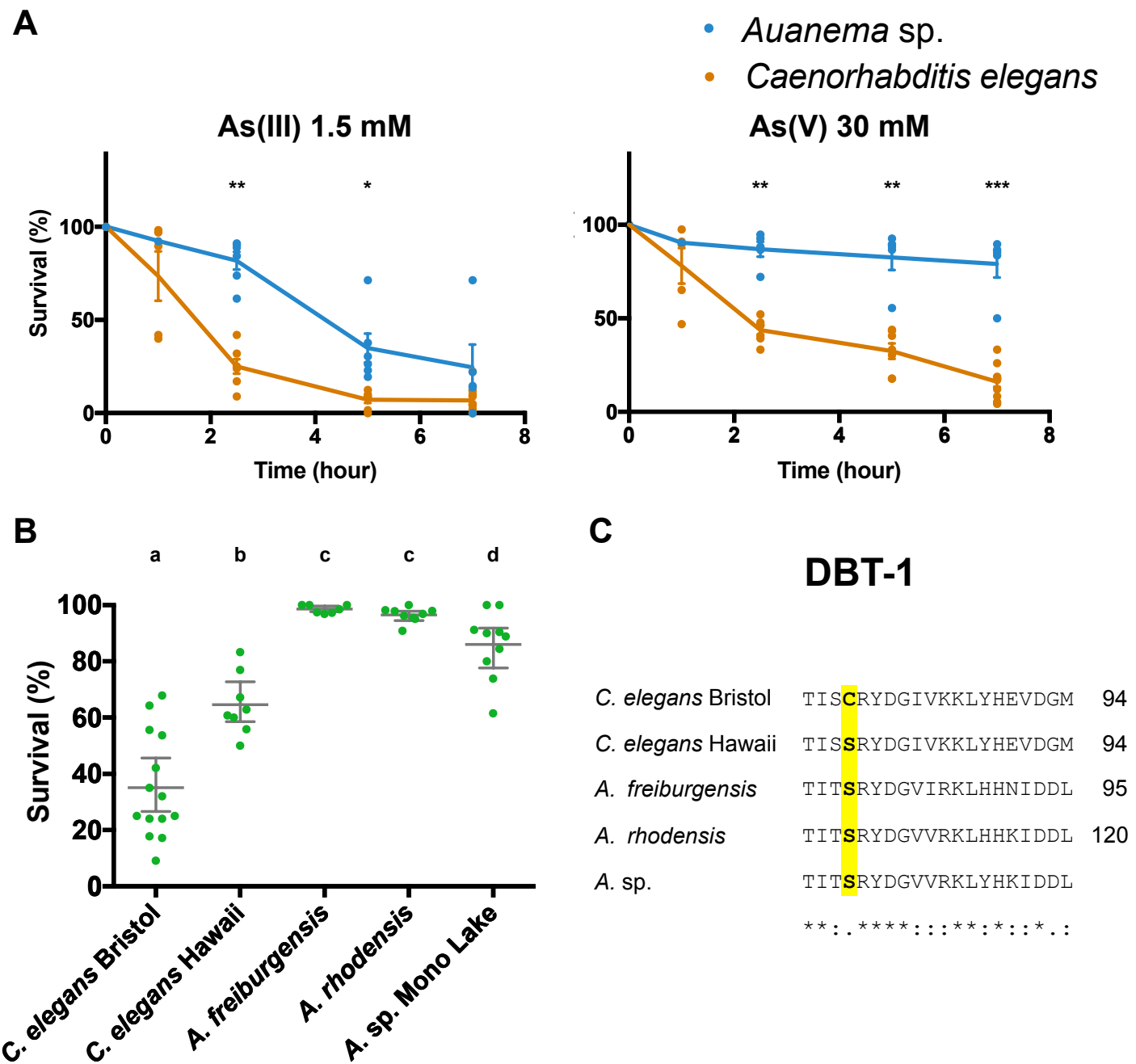
B



C




A
Larva
Vulva
100 µm
B
Larva
Ph
Oc
Embryos
D
P
10 µm


A
Auanema sp.
Caenorhabditis elegans
As(III) 1.5 mM
As(V) 30 mM
Survival (%)
Time (hour)
**
*
**
**
***
B
a
b
c
c
d
Survival (%)
C. elegans Bristol
C. elegans Hawaii
A. freiburgensis
A. rhodensis
A. sp. Mono Lake
C
DBT-1
C. elegans Bristol TISCRYDGIVKKLYHEVDGM 94
C. elegans Hawaii TISSRYDGIVKKLYHEVDGM 94
A. freiburgensis TITSRYDGVIRKLHHNIDDL 95
A. rhodensis TITSRYDGVVRKLHHKIDDL 120
A. sp. TITSRYDGVVRKLYHKIDDL

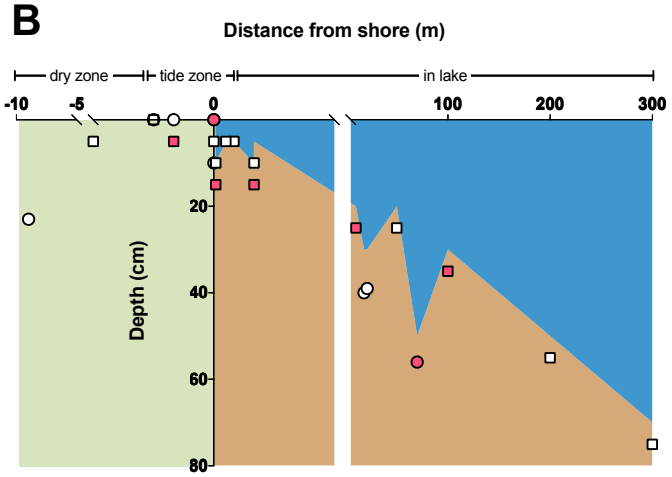
A

"Pristine Beach"
Secluded

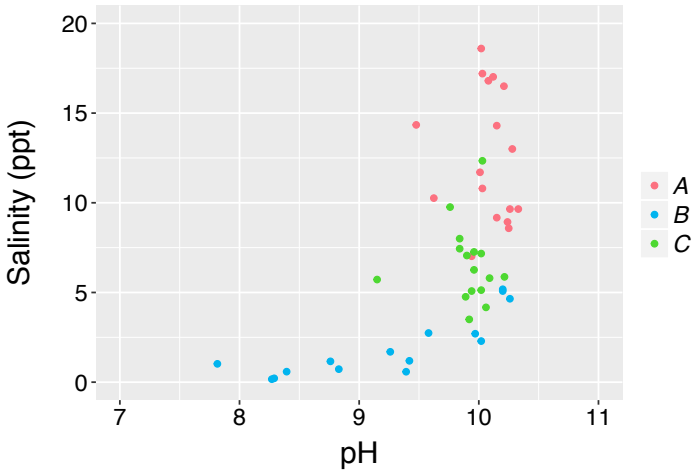
"Navy Beach"
Tufa-rich

"Old Marina"
Rocky shore

B



C

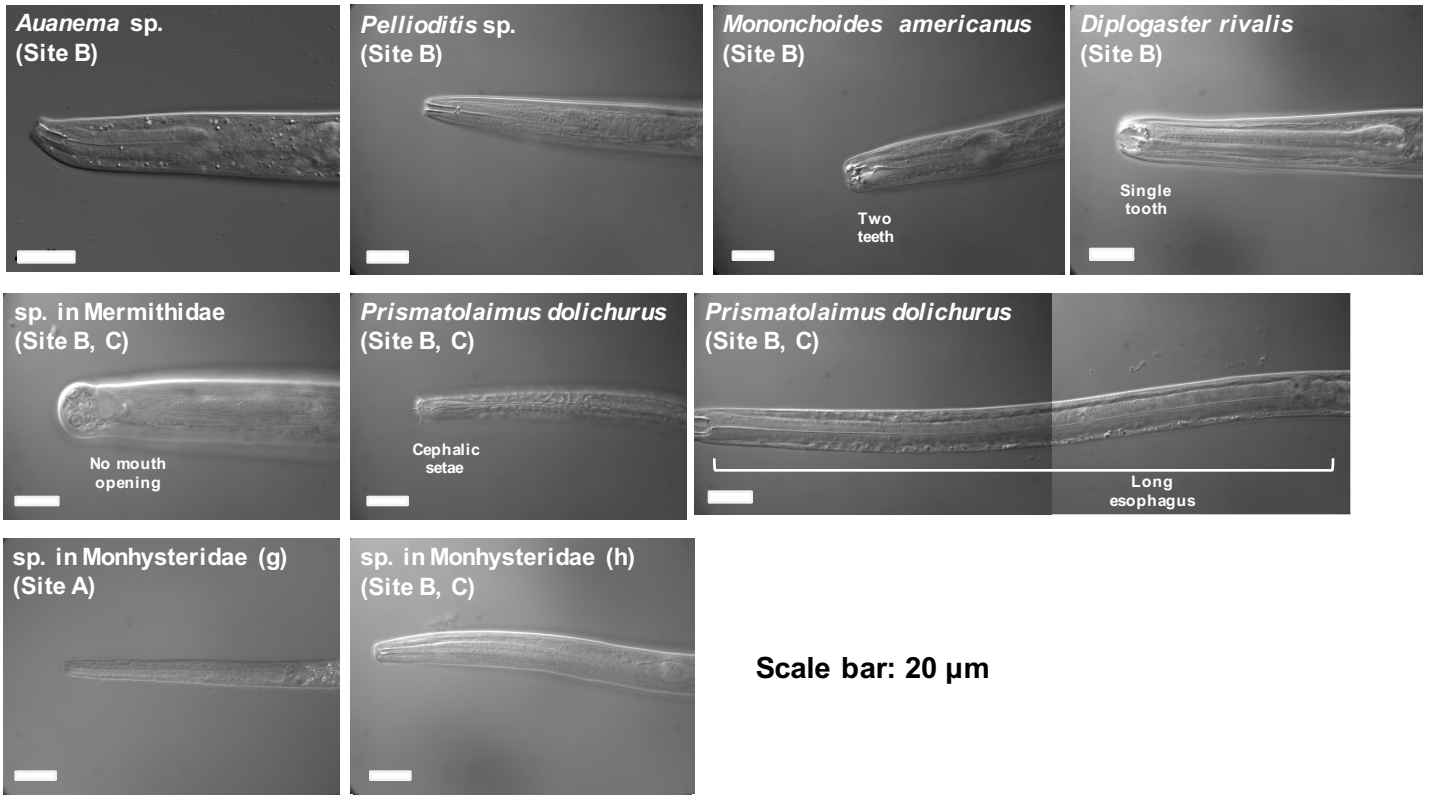


D

Year	Site A			Site B			Site C		
	dry zone	tide zone	lake	dry zone	tide zone	lake	dry zone	tide zone	lake
2016	0/1	1/5	1/3	2/3	9/14	0/1	1/1	0/6	NA
2017	0/1	2/6	3/8	NA	1/3	0/9	6/14	3/4	4/11
Total	0%	27%	36%	67%	59%	0%	47%	30%	36%

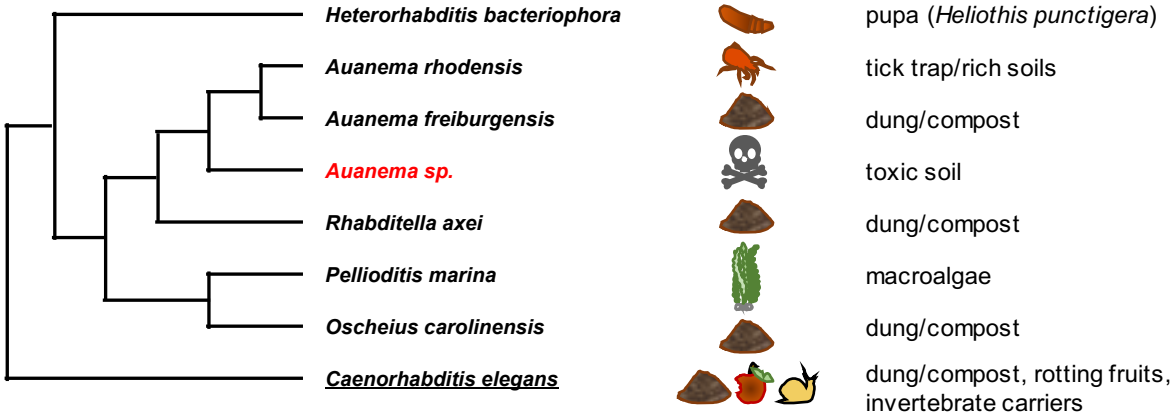
Figure S1. Sample summaries. Related to Figure 1
(A) Pictures of Site A, Site B, and Site C. **(B)** Plot of locations at Site A where samples were collected, relative to the shore (x-axis) and surface (y-axis). Green, land; blue, lake water; brown, sedimentary soil. Circles, samples collected in 2016; squares, 2017. Pink, samples in which nematodes were found. **(C)** Plot of the salinity and pH of measured samples. Each dot represents the measurements from one single sample. Color corresponds to the site where the sample was collected. **(D)** Presence of nematodes at each site. Proportions indicate the number of samples that contained nematodes over the total number of collected samples. Beige, locations where nematodes were isolated in 2016; grey, 2017. NA, non-applicable.

A



Scale bar: 20 µm

B



C

Species	Sequence identity			
	LSU	Closest relative	SSU	Closest relative
Auanema sp. (a)	89%	*A. rhodensis*	96%	*A. rhodensis*
Pellioditis sp. (b)	88%	*Pellioditis* sp.	95%	*Pellioditis* sp.
Mononchoides americanus (c)	90%	*M.* sp.	98%	*M. americanus*
Diplogaster rivalis (d)	92%	*Butlerius* sp.	99%	*D. rivalis*
species in Mermithidae (e)	85%	*Romanomermis culicivorax*	93%	*Mermis nigrescens*
Prismatolaimus dolichurus (f)	99%	*P. dolichurus*	NA	
species in Monhysteridae (g)	NA		92%	*Monhysteridae* sp.
species in Monhysteridae (h)	NA		96%	*Monhysteridae* sp.
species g to species h	NA	--	97%	--

Figure S2. Classification of Mono Lake nematodes. Related to Figure 2
(A) Morphology of species a-h under high magnification. **(B)** Simplified phylogenetic tree showing the relationships of *Auanema* sp. (red) and selected Rhabditina, based on SSU sequences. **(C)** Sequence identity of each isolate, compared to its closest relative. Red, known species.

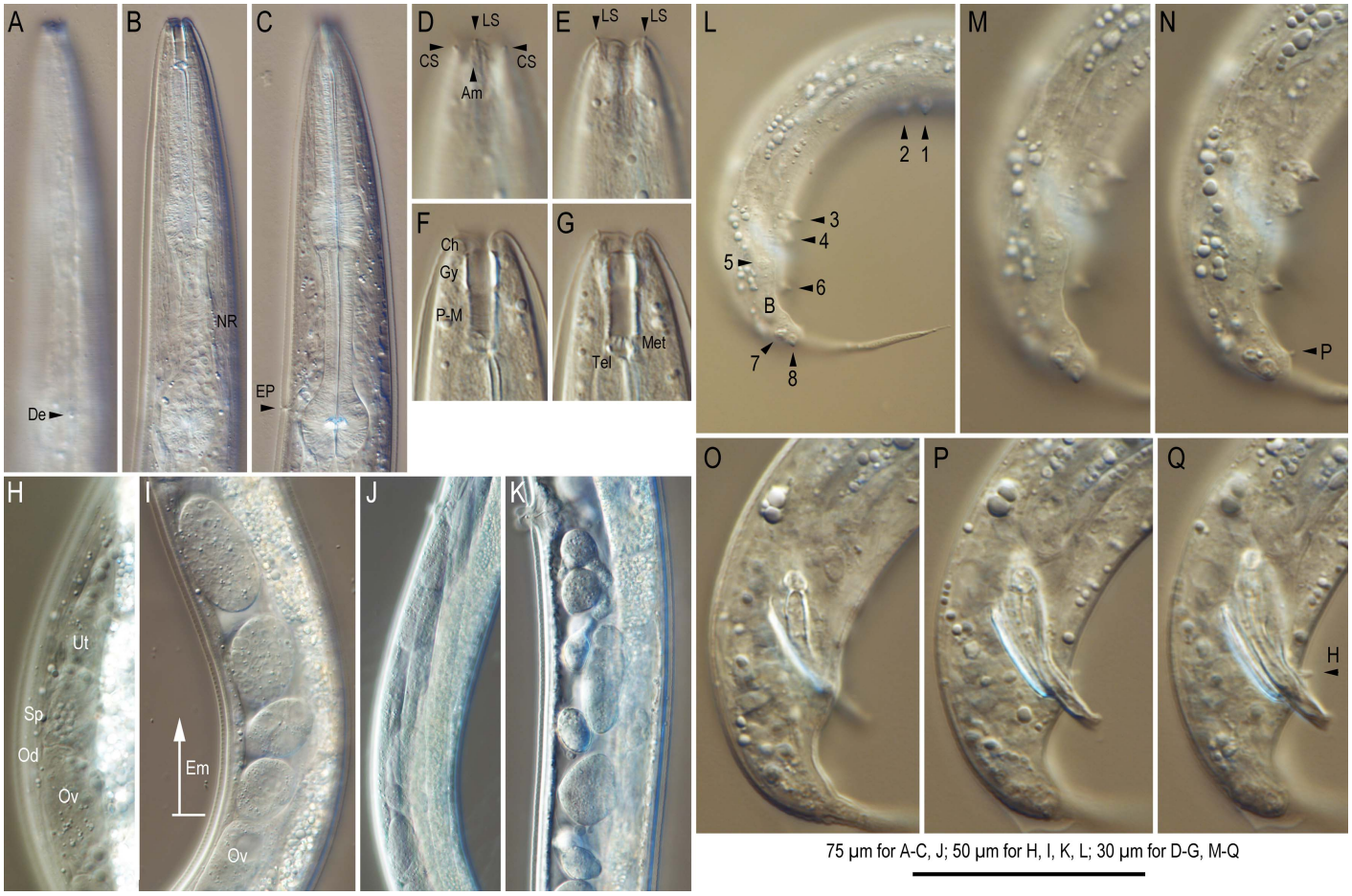
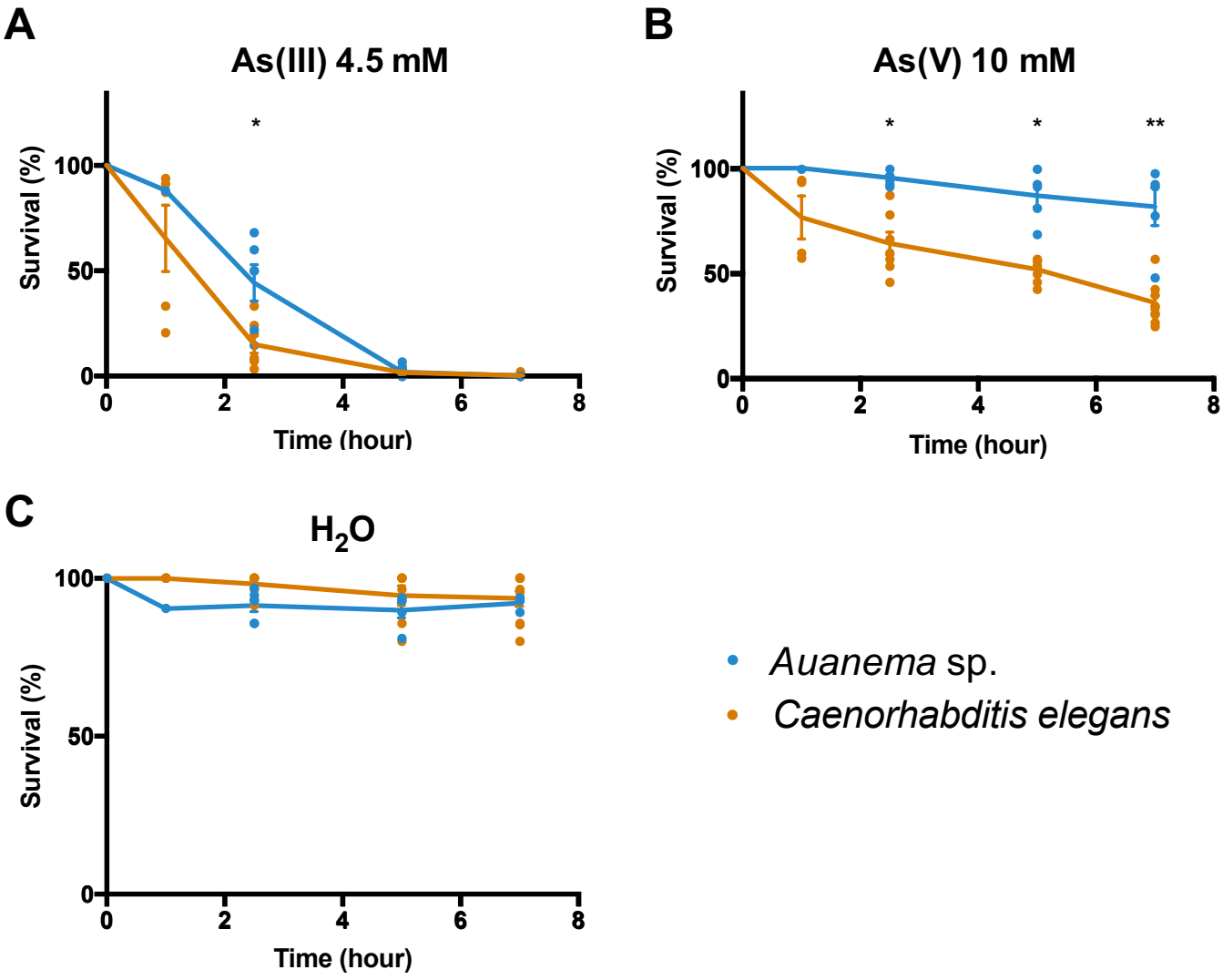


Figure S3. Typological and biological characters of *Auanema* sp. Related to Figure 3
(A-G) Anterior region of *Auanema* sp. in left lateral view. A-C: Anterior region in different focal planes; D-G: Stomatal region in different focal planes. Abbreviations are as follows, De: deirid; NR: nerve ring; EP: excretory pore opening; CS: Cephalic sensilla; LS: labial sensilla; Am: amphid; Ch: cheilostom; Gy: gymnostom; P-M: pro- and mesostegostom; Met: metastegostom; Tel: telostegostom. **(H-K)** Hermaphrodite and female gonads of *Auanema* sp. H: Young hermaphrodite possessing sperm in its spermatheca; I: Young hermaphrodite with developing embryos; J: Mature hermaphrodite with juveniles and developed embryos; K: Unmated mature female with undeveloped oocytes. Abbreviations are as follows, Ut: uterus; Sp: sperm; Od: oviduct; Ov: ovary; Em: embryo. **(L-Q)** *Auanema* sp. male tail in right lateral view. L: Whole tail region; M-Q: Close-up of posterior part in different focal planes. Abbreviations are as follows, numbers: genital papillae; B: bursa; P: phasmid; H: ventral single papilla (hook).



D

```
C. elegans (Bristol)        VQVKEWYVKEGDTISQFDKVCEVQSDKAAVTISCRYDGIVKKLYHEVDGMARVGQALIDV   104
C. elegans (Hawaii)         VQVKEWYVKEGDTISQFDKVCEVQSDKAAVTISSRYDGIVKKLYHEVDGMARVGQALIDV   104
A. freiburgensis (APS7)     VQVKEWYVKEGDTVSQFDNLCDVQSDKAAVTITSRYDGVIRKLHHNIDDLARVGLPLVDI   105
A. rhodensis (APS4)         VQVKEWYVKEGDTVSQFDNLCDVQSDKAAVTITSRYDGVVRKLHHKIDDLARVGLPLVDI   130
A. sp. (PS8402)             VQVKEWYVKEGDNISQFDNLCDVQSDKAAVTITSRYDGVVRKLYHKIDDLARVGQPLVDI
                            ************.:****::*:**********:.****:::**:*:::*.:****  *:*:

C. elegans (Bristol)        EIEGNVEEPEQPKKEAASSSPEAPK-------SSAPKAPESAHSEGKVLATPAVRRIAIE   157
C. elegans (Hawaii)         EIEGNVEEPEQPKKEAASSSPEAPK-------SSAPKAPESAHSEGKVLATPAVRRIAIE   157
A. freiburgensis (APS7)     EIEGEGEEESSNDSKKTE--T-TRETSTSSPSATTQNASEFQGTHGKVLATPAVRRIAME   162
A. rhodensis (APS4)         EIEGEEETTSEAEKPA---ETGASNATSTSSTSSVQSSNDFHAVNGKVLATPAVRRIAME   187
A. sp. (Mono Lake)          EVDDEVAEDSKESKESVSSSVTSPEANATPSPSSVQSSSDFHSTNGKVLATPAVRRIAME
                            *::.:    .. ..       : :       ::. .: :    .**************:*

C. elegans (Bristol)        NKIKLAEVRGTGKDGRVLKEDVLKFL         183
C. elegans (Hawaii)         NKIKLAEVRGTGKDGRVLKEDVLKFL         183
A. freiburgensis (APS7)     NKVDLSAVPATGKEGRVLKEDILKFL         188
A. rhodensis (APS4)         NKVKLSDVPSTGKDGRVLKEDILKFL         213
A. sp. (PS8402)             NKVKLQDVPASGKDGRVLKEDILKSS
                            **:.*  * .:**:*******:**
```

Figure S4. *Auanema* sp. is arsenic resistant. Related to Figure 4
(A-C) Survival curves of *Auanema* sp. (blue) and *C. elegans* (orange) adults in arsenic solutions and water-only control. Points represent individual populations (average 31 animals), bars denote the standard error of the mean. Statistics: non-parametric permutation test at each time point. "*" $q < 0.05$, "**" $q < 0.01$. **(D)** Alignment of DBT-1 protein sequence surrounding the Cys/Ser variant position (yellow).

Sample number	Location	From shore (cm)	Under ground (cm)	Water depth (cm)	Weight of sample (g)	pH	Salinity (ppt)	Nematodes present	Number of nematodes / species
A1	tide zone	0	0	0	368	ND	ND	no	
A2	tide zone	-150	0	0	295	ND	ND	no	
A3	tide zone	0	10	0	350	ND	ND	no	
A4	tide zone	0	0	0	499	9.477	14.342	**yes**	1 / 1
A5	tide zone	-100	0	0	348	ND	ND	no	
A6	dry zone	-900	23	0	216	ND	ND	no	
A7	in-lake	1,800	10	30	367	ND	ND	no	
A8	in-lake	7,000	6	50	680	9.624	10.26	**yes**	~5 / 1
A9	in-lake	2,100	9	30	186	ND	ND	**yes**	ND / 1
A100	tide zone	5	10	0	18.1	10.21	16.5	no	
A101	tide zone	5	5	0	20.7	10.08	16.8	**yes**	1 / 1
A102	dry zone	-300	5	0	41.8	10.12	17.02	no	
A103	tide zone	50	0	5	31.9	10.15	14.3	no	
A104	tide zone	0	5	0	33.1	10.02	18.6	no	
A105	in-lake	30	0	5	26.7	10.01	11.7	no	
A106	tide zone	-150	0	0	27.5	10.03	17.2	no	
A107	tide zone	-100	0	5	21.6	10.03	10.8	**yes**	4 / ND
A108	in-lake	100	5	10	250	10.28	13	**yes**	33 / ND
A109	in-lake	1,000	5	20	241	10.33	9.65	**yes**	1 / 1
A110	in-lake	5,000	5	20	20.8	10.24	8.93	no	
A111	in-lake	10,000	5	30	26.8	10.25	8.58	**yes**	4 / ND
A112	in-lake	20,000	5	50	26	9.94	7.02	no	
A113	in-lake	30,000	5	70	22.8	10.15	9.17	no	
A114	in-lake	100	5	5	26.4	10.26	9.65	no	
B1	tide zone	0	0	0	ND	ND	ND	no	
B2	tide zone	0	0	0	143	ND	ND	no	
B3	in-lake	300	0	10	143	ND	ND	no	
B4	tide zone	0	8	0	318	ND	ND	no	
B5	tide zone	20	10	0	157	ND	ND	**yes**	2 / 1
B6	tide zone	10	10	0	211	ND	ND	**yes**	1 / 1
B7	tide zone	0	5	0	175	9.3925	0.581	**yes**	~15 / 2
B8	tide zone	-100	5	0	231	ND	ND	**yes**	20 / ≥2
B9	tide zone	-100	5	0	162	5.967[a]	0.779	**yes**	~50 / 3
B10	tide zone	0	0	0	248	ND	ND	**yes**	1 / 1
B12	tide zone	0	0	0	330	ND	ND	**yes**	1 / 1
B13	tide zone	-75	8	0	113	ND	ND	no	
B14	tide zone	-75	0	0	311	8.394	0.586	**yes**	~200 / ≥3
B15	tide zone	0	0	0	187	ND	ND	no	
B16	dry zone	-300	6.5	0	222	ND	ND	**yes**	2 / ND
B19	dry zone	-300	5	0	372	ND	ND	no	
B20	dry zone	0	0	0	192	7.815	1.022	**yes**	~20 / 2
B21	tide zone	-120	4	0	133	ND	ND	**yes**	1 / 1
B100	in-lake	30	10	0	32.7	9.42	1.19	no	
B101	tide zone	0	10	0	22	8.83	0.725	no	
B102	in-lake	300	10	0	22.9	8.27	0.166	no	
B103	in-lake	50	10	30	20.3	9.58	2.74	no	
B104	in-lake	10	10	10	24.1	9.97	2.7	no	
B106	in-lake	10	10	10	26.7	8.76	1.16	no	
B107	in-lake	30	10	10	16.7	9.26	1.692	no	
B108	tide zone	-100	10	0	25.5	8.29	0.214	**yes**	40 / 1
B111	tide zone	0	10	0	30.3	10.02	2.29	no	
B112	in-lake	100	10	40	25.5	10.2	5.08	no	
B113	in-lake	300	10	50	31.4	10.26	4.65	no	
B114	in-lake	1,000	10	100	30.9	10.2	5.18	no	
C1	tide zone	0	5	0	168	ND	ND	no	
C2	tide zone	0	0	0	204	ND	ND	no	
C3	tide zone	-30	0	0	245	ND	ND	no	
C4	tide zone	0	4	0	62	ND	ND	no	
C6	tide zone	0	5	0	53	ND	ND	no	
C7	dry zone	-200	5	0	221	10.2145	5.87	**yes**	3 / 2
C8	tide zone	0	3	0	85	9.175	22.361[a]	no	
C100	in-lake	300	0	0	20.8	10.06	4.17	**yes**	1 / 1
C101	tide zone	5	0	0	26.5	9.89	4.76	**yes**	2 / ND
C102	dry zone	-20	0	0	27.9	10.02	7.17	**yes**	8 / 1
C103	in-lake	10	20	0	26.4	10.02	5.13	no	
C104	dry zone	-1,000	0	0	19.7	10.03	12.34	**yes**	52 / ND
C105	in-lake	20	20	20	23	9.92	3.5	no	
C106	tide zone	0	0	0	21.6	9.15	5.72	**yes**	1 / 1
C108	in-lake	100	0	20	19.2	9.94	5.08	no	
C109	in-lake	100	10	0	10.6	9.96	7.26	no	
C110	in-lake	300	10	40	9.2	9.9	7.06	**yes**	2 / ND
C111	in-lake	1,000	0	50	12.5	10.09	5.8	**yes**	2 / ND
C112	in-lake	3,000	10	50	37.2	9.84	7.44	**yes**	1 / 1
C113	in-lake	5,000	0	50	25	9.96	6.26	no	
C114	in-lake	7,000	10	70	18.1	9.84	8	no	
C115	in-lake	10,000	10	100	18.3	9.76	9.76	no	
C130	dry zone	-300	5	0	ND	ND	ND	**yes**	~400 / 1
C131	dry zone	-500	5	0	ND	ND	ND	**yes**	131 / 2
C132	tide zone	-30	5	0	ND	ND	ND	no	
C133	dry zone	-1,000	5	0	ND	ND	ND	**yes**	10 / 1
C134	dry zone	-1,000	15	0	ND	ND	ND	no	
C135	dry zone	-1,000	28	0	ND	ND	ND	no	
C136	dry zone	-500	15	0	ND	ND	ND	**yes**	1 / 1
C137	dry zone	-500	48	0	ND	ND	ND	no	
C138	dry zone	-50	5	0	ND	ND	ND	no	
C139	dry zone	-50	48	0	ND	ND	ND	no	
C140	dry zone	-50	28	0	ND	ND	ND	no	
C141	dry zone	-50	15	0	ND	ND	ND	no	
C142	dry zone	-10,000	2	0	ND	ND	ND	no	
C143	tide zone	-30	5	10	ND	ND	ND	**yes**	1 / 1

Table S1. Soil sample records. Related to Figure 1

Sample numbers indicate the sampling site (A, B, or C) and sampling year (2016 samples start from 1, 2017 samples start from 100). The sign indicates the direction of the sampling: into the lake (positive) or away from the lake (negative). Footnote: a, outliers, excluded from further analysis. ND: not determined.

Species		Site isolated	Date isolated	Isolated alongside	Total number of nematodes from site (all species)	Lifestyle	Clade	Morphology
Auanema sp. (**a**)	PS8402	Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B14**), -75 cm from shore, 0 cm underground, pH 8.394, salinity 0.586 ppt.	August 2, 2016.	Two unidentified nematode species.	~200	Microbe-feeding.	V	Viviparous reproductive mode. Hermaphrodites, females, males observed. Grinder in terminal bulb of the pharynx. Additional descriptions in Suppl. Text.
	PS8403	Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B8**), -100 cm from shore, 5 cm underground.	August 2, 2016.	*Diplogaster rivalis* & species in Monhysteridae.	20			
Pellioditis sp. (**b**)		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B9**), -100 cm from shore, 5 cm underground, pH 5.967, salinity 0.779 ppt.	August 2, 2016.	*Mononchoides americanus* & species in Mermithidae.	~50	Microbe-feeding.	V	Intestine is golden-colored. Grinder in terminal bulb of the pharynx. Tail is long and tapered.
		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), dry zone sediments from close to weeds (**B20**), pH 7.815, salinity 1.022 ppt.	August 2, 2016.	*Mononchoides americanus*.	~20			Males with a brush-like spicule observed.
Mononchoides americanus (**c**)		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B7**), 0 cm from shore, 5 cm underground, pH 9.393, salinity 0.581 ppt.	August 2, 2016.	*Prismatolaimus dolichurus*.	~15	Predatory.	V	Two teeth. Hermaphrodites/females observed. See Chitwood and Chitwood (1937), Calaway and Tarjan (1973).
		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B9**), -100 cm from shore, 5 cm underground, pH 5.967, salinity 0.779 ppt.	August 2, 2016.	*Pellioditis* sp. & species in Mermithidae.	~50			
		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), dry zone sediments from close to weeds (**B20**), pH 7.815, salinity 1.022 ppt.	August 2, 2016.	*Pellioditis* sp.	~20			
Diplogaster rivalis (**d**)		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B8**), -100 cm from shore, 5 cm underground.	August 2, 2016.	*Auanema* sp. & species in Monhysteridae.	20	Predatory.	V	Single tooth. Hermaphrodites/females observed. Two-armed gonad.
species in Mermithidae (**e**)		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B9**), -100 cm from shore, 5 cm underground, pH 5.967, salinity 0.779 ppt.	August 2, 2016.	*Pellioditis* sp. & *Mononchoides americanus*.	~50	Parasitic.	I	No mouth opening observed. Long compared to other Mono Lake nematodes: approximately 4 mm in length. Tail rounded and nubbed.
		Old Marina (37° 59' 12.80" N, 119° 8' 18.70" W), dry zone sediments (**C131**), -500 cm from shore, 5 cm underground.	July 15, 2017.	One unidentified nematode species.	131			Hermaphrodites/females and males observed.
		Old Marina (37° 59' 12.80" N, 119° 8' 18.70" W), dry zone sediments (**C133**), -1000 cm from shore, 5 cm underground.	July 15, 2017.	NA.	10			
Prismatolaimus dolichurus (**f**)		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B7**), 0 cm from shore, 5 cm underground, pH 9.393, salinity 0.581 ppt.	August 2, 2016.	*Mononchoides americanus*.	~15	Predatory.	II	Small teeth-like structures. Prominent cephalic setae. Long esophagus, 2/5 the length of the body.
		Old Marina (37° 59' 12.80" N, 119° 8' 18.70" W), dry zone sediments (**C130**), -300 cm from shore, 5 cm underground.	July 15, 2017.	NA.	~400			Original description de Man, J.G. (1880).
species in Monhysteridae (**g**)		Pristine Beach, (38° 3' 27.91" N, 119° 1' 50.66" W), in-lake sediments (**A9**), 2100 cm from shore, 9 cm underground, 30 cm water depth.	August 2, 2016.	NA.	ND.	Microbe-feeding.	(II, III)	Grinder observed. Adults with single egg in uterus observed.
species in Monhysteridae (**h**)		Navy Beach (37° 56' 21.90" N, 119° 1' 25.93" W), tide zone sediments (**B8**), -100 cm from shore, 5 cm underground.	August 2, 2016.	*Auanema* sp. & *Diplogaster rivalis*.	20	Microbe-feeding.	(II, III)	Grinder in terminal bulb of pharynx. Adults with single egg in uterus observed.
		Old Marina (37° 59' 12.80" N, 119° 8' 18.70" W), dry zone sediments (**C7**), -200 cm from shore, 5 cm underground, pH 10.214, salinity 5.87 ppt.	August 3, 2016.	One unidentified nematode species.	3			

Table S2. Species records. Related to Figures 1 and 2

Lifestyles are predicted by morphology and phylogeny, or observed (*Auanema* sp.) The Clade I-V system by Blaxter is used here.

Supplemental References

S1. Kanzaki, N., Tanaka, S.E., Fitza, K., Kosaka, H., Slippers, B., Kimura, K., Tsuchiya, S., and Tabata, M. (2016). *Deladenus nitobei* n. sp. (Tylenchomorpha: Allantonematidae) isolated from *Sirex nitobei* (Hymenoptera: Siricidae) from Aomori, Japan, a new member of the siricidicola superspecies. Nematology 18: 1199-1217. DOI: 10.1163/15685411-00003025

S2. Herrmann, M., Ragsdale, E.J., Kanzaki, N., and Sommer, R. (2013). *Sudhausia aristotokia* n. gen., n. sp. and *S. crassa* n. gen., n. sp. (Nematoda: Diplogastridae): viviparous new species with precocious gonad development. Nematology 15: 1001-1020. DOI: 10.1163/15685411-00002738

S3. Kanzaki, N., Giblin-Davis, R.M., Gonzalez, R., Wood, L.A., and Kaufman, P.E. (2017). *Sudhausia floridensis* n. sp. (Nematoda: Diplogastridae) isolated from *Onthophagus tuberculifrons* (Coleoptera: Scarabaeidae) from Florida, USA. Nematology 19: 575-586. DOI: 10.1163/15685411-00003071

S4. Kanzaki, N., Kiontke, K., Tanaka, R., Hirooka, Y., Schwarz, A., Müller-Reichert, T., Chaudhuri, J., and Pires da Silva, A. (2017). Description of two three-gendered nematode species in the new genus *Auanema* (Rhabditina) that are models for reproductive mode evolution. Scientific Reports 7: 11135. DOI: 10.1038/s41598-017-09871-1